Mariko NUNO*: **A new lichen substance, Nephroarctin, contained in *Nephroma arcticum***

布 万里子*: ミヤマウラミゴケの新成分，ネフロアークチンについて

(Pl. IX)

Nephroma arcticum (L.) Torss., a beautiful lichen with green algae as gonidia, occurring in northern hemisphere including Japan, was studied chemically at first by Hesse[1], who isolated usnic acid and a hydrocarbon nephrin $C_{20}H_{32}$ and by Zopf[2], who isolated zeorin beside them. In 1960 Wetmore[3] reported the occurrence of a carotenoid as the prominent ingredient together with nephrin (in 58% specimens) and zeorin (in 63% specimens) in this species. As the conclusive factor of his so-called "carotenoid", he showed only crystals yielded under cover glass in G. A. An., but no important property such as over sensitiveness towards air, followed by the fading of color etc.

In the course of my study on lichens collected on higher mountains in Japan, chemisms of *N. arcticum* have called my special attention, as its medullary layer fluoresces by irradiation with ultra violet light, becomes reddish brown by $FeCl_3$ in alcohol, and is colored deep yellow by PD, and its benzene or acetone extract gives characteristic compounds from G. A. An. or G. A. oT. by microcrystallization, suggesting the presence of aldehyde group in its molecule. Recently I have isolated from *N. arcticum* collected on Mt. Ontake, Central Honshu a new lichen substance, which I call **Nephroarctin.**

By the formation of characteristic anilin compound, it was decidedly shown that Wetmore's so-called "carotenoid" represented the crystal form of nephroarctin derivative.

Extraction of Nephroarctin Dried lichen thalli were extracted with ether in a soxhlet apparatus until the extract solution becomes colorless. The orange colored ethereal extract was evaporated and the residue was stirred, after a small amount of warm acetone was added. After the in-

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soluble whitish crude zeorin was filtered off, the combined acetone filtrates were evaporated and the residue was dissolved in boiling benzene. After standing overnight hexagonal crystals of zeorin separated, which were filtrated. The benzene filtrate was moderately concentrated and after standing a little while, filtered again to remove zeorin. The filtrate, concentrated if necessary, was poured into a glass tube percolator, filled with silicagel[5] for column chromatography, which was previously treated with 0.5 N oxalic acid, and at last was separated into 4 fractions by means of eluting solvent, benzene-ethylacetate (5 : 1). Generally these fractions were obtained in the order of usnic acid, nephroarctin, undetermined substance, and zeorin.

Nephroarctin $C_{20}H_{20}O_7$ recrystallized from benzene appears almost colorless or more or less yellowish prisms of m. p. 192–193° and is optically inactive. It is soluble in hot benzene, ethylacetate, acetone, ether and chloroform but scarcely soluble in alcohol.

Color reaction PD + deep yellow; $FeCl_3$ in alcohol + reddish brown; K + yellowish; KC + orange yellow; C in alcohol + yellow; Liebermann's reaction negative; homofluorescein reaction reluctantly positive (successively heating with KOH + chloroform and diluting).

Microcrystallization test Crystals of pure nephroarctin in GE, oT and An are a little different from those of cold benzene extracts of thalli of *N. arcticum*, probably because nephroarctin is mixed with other substances in the benzene extracts. The difference is shown in Table 1 and Pl. IX.

Thin layer chromatography Small fragments of *N. arcticum* were extracted in a small test tube with cold benzene for about 15 minutes. The benzene solution was evaporated and the extract was spotted on glass plates which were coated with Merck's silicagel G and treated with 0.5 N oxalic acid solution, and was chromatographed by using benzene-chloroform-ethylacetate (3 : 3 : 0.3) as developing solvent. After the solvent was evaporated, the chromatograms were detected as colored spots by spraying with 10% sulphuric acid and heating over an alcohol flame. Nephroarctin gives brownish

5) The mixture of 70–80 and 100 meshes (1 : 4) of silicagel for column chromatography was submerged in 0.5 N oxalic acid solution, filtrated after standing overnight, and divided into two parts. The half part of the silicagel was washed with acetone, dehydrated under the reduced pressure and dried in an oven…(A). The other half part of it was washed with water in order to dilute the acidity, before being treated as same as A…(B). A and B which were mixed in 2 : 3 ratio were filled into a glass tube percolator.

Table 1. Results of crystal tests.

	Pure nephroarctin	Cold benzene extract
GE	colorless rectangular or square or rhombic crystals, somewhat resembling those of barbatic acid (Pl. IX, Fig. 1)	colorless granular and small dice like crystals after 1 or 2 days (Pl. IX, Fig. 2)
oT	yellow irregular thin plates, without heating (Pl. IX, Fig. 3)	yellow slightly curved needles after an hour and small thin plates after standing overnight, without heating (Pl. IX, Fig. 4)
An	yellow elongated prisms, swollen in the center and tapering towards each end like a spindle (Pl. IX, Fig. 5)	yellow boat shaped or leaflet-like plates (Pl, IX, Fig. 6)

orange spot at Rf 0.45, whereas zeorin gives a purple spot at Rf 0.045, along with an orange spot of unknown substance at Rf 0.40 and some other pale spots probably yielded by triterpenes.

Chemical constitution of Nephroarctin According to Y. Kuwada, K. Kamiya, and M. Nuno[4], the chemical investigation of Nephroarctin has been carried out on the grounds of infrared and mass spectrometry and through the identification of 10 functional groups by n.m.r. spectroscopy and the determination of the location of each substituents on the two aromatic nucleus by the X-ray crystallography. It was decided to be a phenyl-benzoate derivative ··· 3-methoxy-2, 5, 6-trimethylphenyl 3, 5-diformyl-2, 4-dihydroxy-6-methylbenzoate of the following structure (Fig. 1).

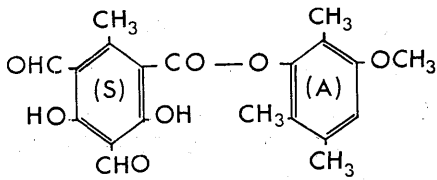

Fig. 1. Chemical structure of nephroarctin.

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versity of Tokyo. Furthermore, I wish to thank Dr. S. Kurokawa, in the National Science Museum, Tokyo, and Mr. M. Togashi, in Res. and Develop. Div., Takeda Chem. Ind., Ltd., for their kindly supplying the valuable samples for extraction.

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Explanation of Plate IX

Figs. 1, 3, 5. Crystals of pure nephroarctin recrystallized in GE (Fig. 1), oT (Fig. 3), and An (Fig. 5). Figs. 2, 4, 6. Crystals of cold benzene extracts recrystallized in GE (Fig. 2), oT (Fig. 4), and An (Fig. 6).

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Nephroma arcticum の化学成分に関する研究は，既に Hesse (1898) や Zopf (1909) によって，ウスニン酸，ネフリン及びゼオリンが報告されているが，近年，Wetmore (1960) は更に UV +, PD +, Fe + の "carotenoid" と称する成分を発表した。

筆者もかねてより，本邦高山で採集せる緑色，美麗な *N. arcticum* の髄層が，PD で鮮黄色を呈するのに注目し，既知の成分と顕微化学的に，或はクロマトグラフィーによって比較するも，いづれも一致するものなく，何か新しい未知のフェノール性の物質で，しかも CHO 基を有する depside か，又は depsidone が予想されたが，Wetmore のいはゆる "carotenoid" もやはり PD + であるので，是非共，筆者の問題としている成分と果して同一のものであるか否かを確認したいと考えて居た。

幸なことに科学博物館の黒川道博士の御好意で，木曾御嶽産の *N. arcticum* を抽出し，未知の地衣成分 nephroarctin を分離することが出来た。更に本成分の化学構造に就いては，武田薬品の桑田胖，神谷和秀博士等の協力を得て，phenyl benzoate の骨格を有する新しい物質である事が明かとなった。

この結果，Wetmore が何故に“carotenoid”と称したかに就いては，その化学的根拠が全く証明されていない為に，甚だ理解に苦しむ。彼の“carotenoid”の唯一の手がかりであるところの An. 塩の顕微鏡写真と，筆者の分離した nephroarctin の An. 塩とを比較すると，黄色の長く伸びた両端は次第に細まり，中央部のやゝ膨らんだ特異な形は非常によく似て居り，その記載並びに呈色反応もよく一致する。しかしながら，nephroarctin は決して“carotenoid”ではなく，むしろ depside に非常に近いものと考えられ，その構造式からは，恰も depside の A 部の COOH が脱炭酸されて脱落してしまったかの如き印象を与へる新しいタイプの物質である。

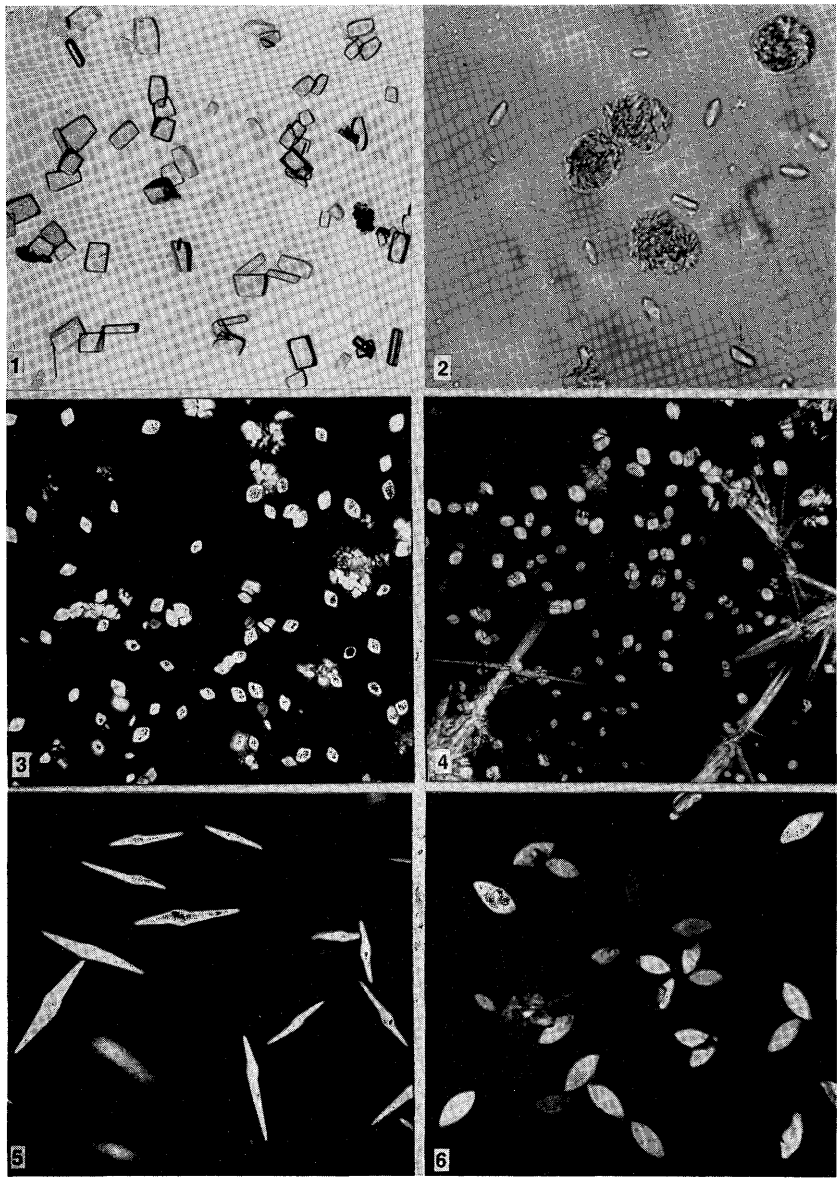
○薬用牡丹の栽培法（佐々木一郎） Ichiro SASAKI: On the cultivation of medicinal Peony, *Paeonia suffruticosa*

奈良県吉野郡には古くから山畑利用の，薬用植物及び果樹の栽培地が沢山ある。薬用のため栽培されて居る牡丹は根を目的とするため根の発育が良く，その皮層が厚く，芳香の強いもので，花としては花弁の数の少ない花の小さな園芸価値の乏しいものである。生薬牡丹皮即ち牡丹の根皮はこの地方が産地で五条，下市等の生薬集荷人によって集荷される。この辺の産出高は日本中で最も多く，品質も生薬界で良いものとされて居る。

薬用の牡丹は 8 月下旬より 9 月初旬（植付後 5～6 年目）畑より掘り取り，根だけは生薬集荷人の所へ出荷され，その根を取った牡丹は 1 本 1 本に分けて苗木とする。（この苗は根が全然付いていない。）すりこぎ同様のこの苗木は，2～3 本を一まとめにしわらで束ねて一株として，9 月初旬～中旬迄に山畑に植え付ける。この時期が牡丹移植の適期で，春は最も移植に不適当の時期である。植え付け株数は 10 アール当り 600～700 株で，植え付けは浅く植え，根元をよく鎮圧し，この挿木同様の苗木には竹で支柱を与えている。これは深く植えると収穫の時，小さい細い根が多くなるため，収量が少なく太い良い根皮が取れないためである。そしてこの植えた苗の根元には晩秋より春迄土を 6～7 cm 位積み上げて置き，春になって寒害の無くなった時出来るだけ早く積み上げた土を除去する。植え付けて 2 年目迄は中耕するが，その後は中耕をしていない。

牡丹を植える際良く気を付けねばならない事は，排水の良いやゝ重い粘質土壌で，傾斜地の東南面で西日のあたる時間の少ない所が良い。連作をとても忌む牡丹は一度植えた土地は 10 年位は植えられないし，前作が芍薬であった所は，牡丹は育たないで枯死する株が多いので良くない。苗木は乾燥しない中になるべく早く植える方が良いが，日時を要する時は植える迄日陰で時々水をかける程度で決して永く水に漬けてはいけない。水に漬けた苗木は畑に植えた時活着が悪いので注意する必要がある。

（津村研究所）



M. NUNO: *Nephroma arcticum*